

Human Papilloma Virus Prevalence and Types among Iranian Women Attending Regular Gynecological visits

Shafaghi B^{*1}, Jarollahi A², Yousefzadeh B², Ameri A³, Moghadam S³, Mostafavi M²

1. Department of Pathology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2. Pars Hospital Laboratory, Tehran, Iran.

3. Department of Radiation Oncology, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*Corresponding Author: Shafaghi B

Email: behshaf@hotmail.com

Abstract

Introduction: Human papilloma virus prevalence data is scarce in Iran. This study was performed to evaluate type-specific human papilloma virus prevalence and to compare it with Pap smear results among Iranian women attending regular gynecological visits.

Patients and methods: A total of 851 women aged 18-65 years, attending regular gynecological visits were retrospectively evaluated. Human papilloma virus detection and genotyping was performed using polymerase chain reaction technique. Cytological evaluation was performed by Papanicolaou method and the association between cytological results and human papilloma virus profile was analyzed.

Results: Nineteen different types of human papilloma virus were detected in 265 of 851 patients (31.1%). Overall infection as well as infection with high risk human papilloma virus types; were highest in women aged 18-25 years and decreased with age. Type-specific prevalence of human papilloma virus -16 and 18 was 7.3% and 2.8% respectively. There was also an upward trend in the prevalence of high risk human papilloma virus infection as the abnormality in cytology increased. The prevalence of human papilloma virus related events was 29.1% among virus positive patients and declined from low grade squamous intraepithelial lesion (18.2%) to high grade squamous intraepithelial lesion (3.9%).

Conclusion: Our study indicated that the burden of human papilloma virus infection among Iranian females was higher in comparison with previous estimates reported from Iran. Furthermore, higher prevalence of premalignant changes in Iranian women infected with high risk human papilloma virus types other than vaccine types should be considered in immunization programs and development of population-specific human papilloma virus vaccines.

Key words: Human papilloma virus, prevalence, type, cervical cancer, Iran.

Introduction

Human Papilloma Virus (HPV) infection is known as one of the most common sexually transmitted infections worldwide. HPV is also recognized as the leading cause of cervical cancer which is the second most common cancer in women aged 15 - 44 years⁽²⁾. Iran has a population of more than 25 million women aged 15 years and older, who are at risk of cervical carcinoma. Of those women 643 are diagnosed with cervical carcinoma and 286 women die from this disease every year. Cervical cancer with an age-standardized incidence rate of 2.2

per 100,000 women per year, ranks as the second most common malignancy of female reproductive system in Iran⁽³⁾. Human papilloma viruses belong to papillomaviridae family⁽⁴⁾. So far, 118 types of these viruses have been identified, among them about 40 HPV viral types are commonly found in the genital tract^(5, 6). According to the oncogenic potential, genital HPV types have been subdivided into low-risk and high-risk types. High Risk HPV (HR-HPV) types (including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) are involved

in cervical carcinogenesis, whereas Low Risk HPV (LR-HPV) types, such as HPVs 6 and 11, mainly cause benign lesions such as condyloma acuminatum^(2, 8). HPV-16 and 18 are the most prevalent types reported worldwide⁽⁹⁾. The high frequency of HPV-16 and HPV-18 in cervical cancers has led to development of HPV vaccines (Gardasil and Cervarix) against HPV 6, 11, 16 and 18 which have the potential to reduce the incidence of cervical cancers^(2, 10). Furthermore, HPV DNA testing is used as an adjunct to Pap smear test in the diagnosis of cervical cancers and acts as a guide in approaching those patients with borderline pathology^(11, 12). HPV DNA Polymerase Chain Reaction (PCR) is now recommended for patients with cytological abnormalities⁽¹³⁾.

Indeed, HPV prevalence data is a basic need to predict the potential advantages of HPV immunization and develop more reliable cervical cancer screening tests^(11, 12). Type-specific HPV prevalence has been shown to vary among populations according to age, region and the type of population^(9, 14, 15). Varied distribution of HPVs across populations indicates the importance of HPV surveys in different geographical regions. Unfortunately, HPV prevalence data is not yet available for the general population of Iran and published data in this field is limited⁽³⁾. This study was performed to provide information on HPV prevalence and types among Iranian women attending regular gynecological visits and to compare it with Pap smear results.

Patients and methods

A total of 912 consecutive women attending the gynecology outpatient clinic of Pars hospital, Tehran, Iran, for general genital symptoms from January 2010 to June 2012 were retrospectively analyzed. Information on past medical history and sexual behavior was obtained during visit by a gynecologist. Patients were considered eligible to enter the study if they had current or past sexual activity and no previous diagnosis of cervical cancer. No one was known as to be a sexual worker or HIV positive patient. Because of the insufficient data, 61 cases were excluded from the study and finally the data of 851 women were used for final evaluation.

In order to prepare thin prep slides, cervix epithelial cells were obtained by use of an

endocervical cytobrush and placed into microtubes containing 500 micro liter of Phosphate-Buffered Saline (PBS). All smears were covered with cover slips and screened by a pathologist, after fixation in alcohol and Papanicolaou staining. According to the Bethesda 2001 system, the results were classified into these categories: Within Normal Limit (WNL), Atypical Squamous Cells of Undetermined Significance (ASCUS), Low Grade Squamous Intraepithelial Lesions (LSIL), and High grade Squamous Intraepithelial Lesions (HSIL)⁽¹⁶⁾.

DNA extraction from Pap-smears

By soaking the slides in xylene, coverslips were removed in two days. Then, the slides were destained using several alcohol solutions graded down to fifty percent. After scrapping the cells from the slides by the blade, the cells were transferred into a vial contained 200 ml of PCR buffer. Digestion of cells was performed by adding 20mg of proteinase k. DNA was precipitated after extracting digested cells by phenol-chloroform (3 times) and chloroform (once) and adding sodium acetate and ethanol. Subsequently, the obtained products were centrifuged at 14,000 rpm for forty minutes. Precipitated DNA samples were dried and dissolved in 50cc units of TE (0.1mM EDTA, 10mM Tris-HCl [pH 7.5])⁽¹⁷⁾.

HPV DNA detection and genotyping

HPV DNA was detected by PCR using MY09/MY11 (as outer) and GP5+/GP6+ (as inner) primers with the following sequences [de Roba Husman et al.:1995 modified in German Cancer Research Center]: MY09 5'CGTCCMARRGGAWAC TGATC 3', MY11 5'GCMCAGGGWCATAAYAATGG 3', GP5 + 5' TTTGTTACTGTGGTAGATACTAC and GP6 + 5' GAA AAA TAA ACT GTA AATCATATTC, designed to amplify a segment of nearly 450bp of the L1 region of HPV genome⁽¹³⁾. In order to verify the intactness of DNA, a 167 bp fragment of b-globin gene was also amplified as a control. The amplified fragments were analyzed by 2% agarose gel electrophoresis⁽¹⁸⁾.

HPV typing was performed by using Restriction Fragment Length Polymorphisms (RFLP). MY09/MY11 PCR products were used for restriction digestion. The restriction endonucleases for RFLP analysis of L1 region of HPV genome include PstI, HaeIII, DdeI and RsaI. Reactions occurred at 37° C for 60 minutes. Digested products were

electrophoretically resolved on 3% agarose gels and stained with ethidiumbromide. HPV types were identified by comparison of the obtained RFLP pattern with those available in GenBank database ⁽¹⁹⁾.

Statistical Analysis

The data were analyzed using SPSS 14 program. The distribution of HPV infection according to age and cytological lesion types was analyzed (descriptive analysis). Chi-square test was used to compare the prevalence between different groups. Statistical significance was defined as $p < 0.05$.

Results

Nineteen different HPV types were detected in 265 of the 851 specimens (31.1%). All detected HPV types were stratified into following four categories: HPV16, HPV18, HR- HPV types other than 16 and 18 (including: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and Low Risk HPV types (including: 6, 11, 42, 43, 44). The distribution of HPV types was analyzed according to age and cervical cytology in infected women. The women were classified by their age into following age groups: 18-25 years ($n=61$), 26-35 ($n=514$), 36-45 ($n=171$) and >45 ($n=105$). The mean age of 851 studied women was 31.6 years (range 18–65). Table 1 displays the distribution of HPV genotypes in infected women by age.

HPV infection prevalence was highest among young women and decreased with age. Women aged 18-25 years had the highest prevalence of HPV (57.4%), followed by women aged 36-45 years with prevalence of 34.6%. Irrespective of the age,

the HR-HPV types were the most frequent (81.5% of overall HPV prevalence) and type-specific prevalence of HPV-16 and HPV-18 were 7.3% and 2.8%, in order. The highest HR-HPV prevalence (57.4%) was seen in younger females (18–25 years), and the prevalence was 8.6% among females aged greater than 45 years. The prevalence of HPV co-infections was 29.1% (77 out of 265 positive samples). There was a peak in HPV co-infection rate among women aged 18-25 years (36.1%) with a second peak among oldest age group (33.3%).

Table 2 presents the distribution of HPV types in infected women in relation to cytological results. Among 265 HPV positive women, 101 cases (38.1%) had abnormal cytology in their Pap smears. Greater than 95% of women with abnormal cytology were infected with HR-HPV types, whereas less than 5% of them were infected only with LR-HPV types. The prevalence of ASCUS, LSIL and HSIL was 21.8%, 17.6% and 5.5% in patients infected with HR HPV types and 4.1%, 4.1% and 0.0% in patients infected only with LR HPV types, respectively. The prevalence of HR HPV types among HPV-positive samples was 95.9%, 95.0% and 100% in patients with ASCUS, LSIL and HSIL, respectively. No invasive cervical cancer was detected among HPV-positive women.

Table 2 also shows that a significant lower proportion of abnormal cytology was detected among women infected by HPV-16 or HPV-18 in comparison with women infected by the other HR-HPV types. Seventy seven samples (29.1% of all HPV positive samples) were infected with multiple HPV types which included at least one HR-HPV

Table 2: Distribution of HPV types in relation to cytological results.

Infection Group	Genotype Risk	WNL	ASCUS	LSIL	HSIL	Total (%)
HPV Positive		164(61.9%) ^c	49(18.5%) ^c	40(15.1%) ^c	12(4.5%) ^c	265
HPV31,33,35,39,45,51,52,56,58,59,66,68	HR	102(59.4%) ^d	35(20.3%) ^d	26(15.1%) ^d	9(5.2%) ^d	172(64.9%) ^c
HPV6, 11, 42, 43, 44	LR	69(64.5%) ^d	19(17.8%) ^d	16(14.9%) ^d	3(2.8%) ^d	107(40.4%) ^c
HPV 16	HR	28(45.2%) ^d	15(24.2%) ^d	16(25.8%) ^d	3(4.8%) ^d	62(23.4%) ^c
HPV 18	HR	13(54.2%) ^d	8(33.3%) ^d	3(12.5%) ^d	0(0.0%) ^d	24(9.1%) ^c
HR-HPV Positive	HR	119(55.1%) ^d	47(21.8%) ^d	38(17.6%) ^d	12(5.5%) ^d	216(81.5%) ^c
Only LR-HPV Positive	LR	45(91.8%) ^d	2(4.1%) ^d	2(4.1%) ^d	0(0.0%) ^d	49(18.5%) ^c
Co-infection	HR	41(53.2%) ^d	19(24.7%) ^d	14(18.2%) ^d	3(3.9%) ^d	77(29.1%) ^c

HR: High Risk; LR: Low Risk; WNL: Within Normal Limit; ASCUS: Atypical Squamous Cells of Undetermined Significance; LSIL: Low grade Squamous Intra-Epithelial Lesion; HSIL: High-grade Intra-Epithelial Lesion; c: percentage in respect to all positive samples; d: percentage in respect to positive samples in group.

type. HPV-16 was the most common type found in co-infection cases. HPV co-infection rate peaked in women with ASCUS (38.8%) and declined from LSIL to HSIL (35.0% to 25.0%).

Discussion

To our knowledge, this study represents the largest study of HPV prevalence in Iranian women which includes HPV prevalence data from 851 women in Tehran.

In most previous studies on HPV prevalence in Iran, some inclusion criteria such as normal/abnormal cytology in Pap smears or the presence of cervical carcinoma has been used. In one study in northern Iran, carried out by Ghaffari et al. ⁽²⁰⁾, after cytological evaluation of 127 women, HPV genotyping was performed in all infected samples using PCR with specific primers for HPV 16, 18, 31, 33, 11 and 6. Among infected samples, HPV-16 had the highest prevalence (76%), followed by HPV-18 (12.7%) and HPV11,6 (8.5%). In a similar study in northern Iran, Hamkar et al. ⁽²¹⁾ analyzed

100 cervical biopsy specimens and reported HPV infection rate of 64.3% in women with abnormal cervical cytology and 9% in women with normal cervical cytology. HPV-16 and 18 were the most commonly detected types (55.6%), followed by HPV-6 and 11(22.3%) in abnormal cytology group.

In comparison with these studies, higher overall prevalence of HPV infection (31.1%) as well as the higher prevalence of HR-HPV types other than 16 and 18 (20.2%) in our study may arise from using different inclusion criteria and also a different population profile with a high rate of women with abnormal cervical cytology. Our data on HPV-16 and 18 prevalence (7.3% and 2.8%, respectively) was similar to what reported worldwide. For instance, in a review by Smith et al. ⁽¹⁴⁾, type-specific prevalence of HPV 16 or 18 was found to be less than 8% and HPV-16 infection rate was reported in less than 25% of overall HPV infections in lower sexual risk population. HPV-16, with different prevalence rates, is the most common viral type worldwide. The second most common type is HPV-

Table1: Distribution of HPV genotypes in infected women by age

Age Group	18–25 years	26–35 years	36–45 years	>45 years	Total
Number of Samples (%)	61(7.2%) ^t	514(60.4%) ^t	171(20.1%) ^t	105(12.3%) ^t	851(100%) ^t
HPV negative	26(42.6%) ^a	336(65.4%) ^a	128(74.8%) ^a	96(91.4%) ^a	586(68.9%) ^t
HPV positive	35(57.4%) ^a	178(34.6%) ^a	43(25.2%) ^a	9(8.6%) ^a	265(31.1%) ^t
HPV31, 33, 35 ,39, 45 ,51, 52, 56, 58, 59, 66, 68	27(77.1%) ^b	113(63.5%) ^b	25(58.1%) ^b	7(77.8%) ^b	172(20.2%) ^t
HPV 6, 11, 42, 43, 44	15(42.8%) ^b	71(39.9%) ^b	15(34.9%) ^b	6(66.7%) ^b	107(12.6%) ^t
HPV 16	8(22.8%) ^b	49(27.5%) ^b	3(7.0%) ^b	2(22.2%) ^b	62(7.3%) ^t
HPV 18	7(20.0%) ^b	14(7.9%) ^b	1(2.3%) ^b	2(22.2%) ^b	24(2.8%) ^t
HR-HPV positive	29(82.9%) ^b	150(84.3%) ^b	32(74.4%) ^b	5(55.6%) ^b	216(25.4%) ^t
Only LR-HPV positive	6(17.1%) ^b	28(15.7%) ^b	11(25.6%) ^b	4(44.4%) ^b	49(5.8%) ^t
Infection with single HPV type	22(62.9%) ^b	122(68.5%) ^b	38(88.3%) ^b	6(66.7%) ^b	188(22.1%) ^t
Co-infection with multiple HPV types	13(36.1%) ^b	56(31.5%) ^b	5(11.7%) ^b	3(33.3%) ^b	77(9.0%) ^t

HR: High Risk; LR: Low Risk; a: percentage in respect to all samples in the age group; b: percentage in respect to all HPV positive samples in the age group; t: percentage of total.

18 in Europe, Central and South America, HPV-52 and 58 in Asia and HPV-52 and 53 in North America^(9, 15, 22, 23). However, there were a few exceptions, such as one study in Benin in West Africa⁽²³⁾ which reported HPV-59 as the most common type detected and another study in USA⁽²⁴⁾ in which HPV-84 and HPV-62 were the most detected ones.

Moreover, in most population-based studies, HR-HPV types were detected in greater than 70% of all HPV positive patients. In our study, 81% of infected women presented HR viral types, whereas this rate was 75% in Brazil⁽²⁵⁾, 76% in Korea⁽²⁶⁾ and 88% in Benin, in West Africa⁽²³⁾.

Our data also show a significant lower proportion of abnormal cytology among women infected by HPV-16 and/or HPV-18 (vaccine HPV types) in comparison with the ones infected by other HR viral types which indicates the importance of considering other HR-HPV types in immunization and screening policies in Iran.

Based on our results, there was a peak in HPV prevalence in women aged 18-25 years (57.4%) with a downward trend as age increased. The trend is similar to what has been reported in most other world regions which indicates a higher possibility of getting new infections in younger age groups^(14, 15, 24, 27, 28, 29). However, we believe, it may be related to the current changes in sexual habits among young Iranian females⁽²²⁾. The prevalence of HPV co-infections was 29.1% (77 out of 265 positive samples). There was a peak in HPV co-infection rate among women aged 18-25 years (36.1%) with a second peak among oldest age group (33.3%).

According to our results, as cytological abnormality increased, there was an upward trend in overall prevalence of HPV infection as well as prevalence of HR-HPV types. The prevalence of HR-HPV types among HPV positive samples was 95.9%, 95.0% and 100% in patients with ASCUS, LSIL and HSIL, respectively. Similarly in a study by Delgado et al.⁽²⁷⁾ in Spain, HR-HPV prevalence among HPV positive samples was 94.3%, 78.1% and 100% in ASCUS, LSIL and HSIL/CC cases, respectively. Also in another study in Benin, in West Africa⁽²³⁾, 96% of HPV positive women with both abnormal cytology and HPV infection were infected with HR-HPVs. Regarding the high presence of HR-HPVs in precancerous lesions, HR-HPV positive women with normal cytology may benefit from more frequent

follow-ups⁽³⁰⁾.

Moreover, about 29% of all HPV positive samples were infected by multiple HPV types, in which HPV-16 was the most prevalent. HPV co-infection rate peaked in women with ASCUS and declined from LSIL to HSIL. Interestingly, in most similar studies the highest co-infection rate was reported in women with cervical cytology of ASCUS⁽³¹⁾ or LSIL^(27, 32). It seems, as precancerous changes progress, one potent HPV type dominates the others⁽²⁾.

Our study had several limitations: first, HPV prevalence results reported here were entirely limited to our population profile and may not represent those of the general population as we studied sexually active women with general genital symptoms referred to our outpatient gynecology clinic. The second limitation was that, although our study indicated a remarkable higher prevalence of HR HPVs other than HPV-16 and 18 in comparison with previous studies in Iran, type-specific HPV prevalence of those types was not determined independently. The third limitation was that this study did not include HPV prevalence analysis among patients diagnosed with invasive cervical cancer; and the fourth limitation was that since many HPV infections clear spontaneously, prevalence results presented in our study underestimates the cumulative HPV incidence^(24, 33, 34).

Conclusion

Our study indicated that the burden of HPV infection among Iranian females was higher in comparison with previous estimates reported from Iran. Furthermore, since HPV vaccines are not introduced in Iran yet, higher prevalence of premalignant changes in women infected with HR-HPV types other than vaccine HPV types, need to be considered in immunization programs and development of population-specific HPV vaccines. The remarkable difference in prevalence of HPV types among previous studies, confirms the need to further investigate the epidemiology of HPV infection in Iran.

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